Attoney's Docekt No.: 20241/0203481-US0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Nobuo Mochizuki, et al.
Application No.: 10/553, 108
Filed: October 12, 2005
Confirmation No.: 8647
Art Unit: 1626
Examiner; Havlin, Robert H.
For: PHENYLAZOLE COMPOUND, PRODUCTION PROCESS THEREFOR AND ANTIOXIDANT

DECLARATION UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Seiichi Uchida, hereby declare and state that:

1. I am a citizen of Japan, residing at 524-17, Higashi-koiso Oiso-machi, Naka-gun, Kanagawa, 255-0004, Japan.

2. I am one of the inventors of the subject application, and I am fully familiar with the subject matter thereof as well as the references relied upon by the Examiner in the prosecution of this application.

3. I obtained a Master's degree from in pharmaceutical sciences from Nagoya City University in March, 1982, where I studied the zymology of the lysosomal ATPase of chicken's liver.

4. I am currently employed by Nippon Soda Co., Ltd., and began working for Nippon Soda Co., Ltd., in April, 1982, whereat I have been engaged in research on drug discovery.

5. In order to demonstrate the difference in effects on the eye subjected to light irradiation between the present invention and the cited reference, namely US Patent No.

6,342,516 by Umeda, et al., I conducted the following comparative test using Compound 37 disclosed in the present specification and Compounds 3-19 disclosed in the cited reference (hereinafter, abbreviated as "516" patent).

METHOD

Preparation of samples

Compound 37 and Compounds 3-19 were suspended in 1% methyl cellulose solution, respectively.

As a Control Sample, 1% methyl cellulose solution was prepared.

Pretreatment on Mouse

16 male Slc:BALB/cCr mice (aged 5 weeks) were dark-adapted by keeping the mice in a darkened room for 48 hours.

Administration of samples

Compounds 37 and 3-19 were administered orally to two mice, respectively, at a dose of 30 mg/kg or 100 mg/kg.

The Control Sample was administered orally to eight mice at a dose of 10 ml/kg of 1% methyl cellulose solution.

Light exoposure

Immediately after the administration, a mydriatic (Mydrin manufatured by Santen Pharmaceutical Co., Ltd.) was instilled into eyes of four mice to which Control Sample was administered (referred to as an irradiated control group), two mice to which Compounds 37 was administered at 30 mg / kg (referred to as a 30-mg/kg Compound 37 group), two mice to which Compound 37 was administered at 100 mg / kg (referred to as a 100-mg/kg Compound 37 group), two mice to which Compound 3-19 was administered at 30 mg / kg (referred to as a 30-mg/kg Compound 3-19 group), and two mice to which Compound 3-19 was administered at 100 mg / kg (reffered to as a 100 mg/kg Compound 3-19 group). 30 minutes after the dosing, the mice were exposed to 5,000 to 6,000 lux of diffuse, white fluorescent light for 2 hours. In order to maintain a constant level of the exposure intensity at every cage, the exposure position was changed at every 25 minutes.

The residual four mice to which Control Sample was administered were exposed to the natural light (at approximately 20 lux) as an unexposured control group.

Four days after the exposure, the mice were anesthetized and exsanguinated

exposed to the natural light (at approximately 20 lux) as an unirradiated control group.

Four days after the irradiation, the mice were anesthetized and exsanguinated from the abdominal aorta to result in death. The right eye of the respective mice was isolated by passing a black thread through the conjunctiva at the position of twelve o'clock in the eyeball, and then immersed in a fixing solution containing 4% by weight of formaldehyde and 0.25% by weight of glutarate aldehyde.

Hematoxylin-eosin staining

The cornea was removed three hours after the dissection. The fixing solution was replaced with a 10% formalin neutral buffer 24 hours after the cornea was removed. Then, serial sections of the retina were prepared so that one of the serial sections includes the optic papilla. The serial sections were stained with hematoxylin-eosin.

Evaluation

The respective thickness of the outer nuclear layer of the retina was measured at 5 points with the respective intervals of 200 $\mu$m above the optic papilla. Then, the area of the outer nuclear layer with a width of 1,000 $\mu$m above the optic papilla was calculated as the sum of five areas of trapezoids, the respective area or the trapezoids being calculated in accordance with the following formula:

(sum of the thicknesses ($\mu$m) of the outer nuclear layer measured at both ends of 200-$\mu$m-intervals) $\times$200 ($\mu$m) $\div$ 2.

With respect to the unirradiated control group and the irradiated control group, the calculated areas of each four mice were averaged to calculate the recovery rate (%) as described below. With respect to the 30-mg/kg Compound 37 group, the 100-mg/kg Compound 37 group, the 30-mg/kg Compound 3-19 group, and the 100-mg/kg Compound 3-19 group, the calculated areas of each two mice were averaged to calculate the recovery rate (%) as described below.

The recovery rate (%) of the respective group to which Compound 37 or 3-19 was administered at 30 or 100 mg/kg was calculated in accordance with the following formula:

$$\text{Recovery rate (\%)} = 100 - \frac{\text{(the area of the unirradiated control group) - (the area of the respective gorup)}}{\text{(the area of the unirradiated control group) - (the area of the irradiated control group)}} \times 100$$

(wherein the respective group represents the 30-mg/kg Compound 37 group, the 100-mg/kg Compound 37 group, the 30-mg/kg Compound 3-19 group, or the

RESULTS

Results are shown in the following table.

| | Recovery rate (%) |
|---|---|
| 30-mg/kg Compound 37 group | 18.6 |
| 100-mg/kg Compound 37 group | 45.0 |
| 30-mg/kg Compound 3-19 group | -1.1 |
| 100-mg/kg Compound 3-19 group | 5.1 |

As is apparent from the results, Compound 37 according to the present invention significantly increased the recovery rate in comparison with Compound 3-19 disclosed in "516" patent.

CONCLUSION

The above-mentioned results shows that Compound 37 according to the present invention unexpectedly exhibits protective effects on the retina against photodamage.

6. I fully understand the content of this declaration.

7. I, Seiichi Uchida, the undersigned declarant, declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both, under section 1001, of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 14 day of January, 2010.

Seiichi Uchida

(Seiichi Uchida)